EFFECT OF LEAF AGE ON PHOTOSYNTHETIC ASSIMILATION OF CARBON DIOXIDE IN TEA PLANTS

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One of the requisites for continuous production of flush by tea plants is a supply of reduced carbon compounds such as carbohydrates. These carbon compounds originate almost entirely from photosynthetic assimilation of atmospheric carbon dioxide by the green parts, primarily the leaves, of tea plants. While the validity of the above statements is universally accepted, very little is known of the potential of tea leaves to assimilate atmospheric carbon dioxide photosynthetically. As a result of this, the contribution of the various leaves on a tea bush to its nutrition is at present largely a matter of guess-work. The present investigation was carried out for the purpose of increasing our knowledge on this subject.

Detached tea leaves were allowed to photosynthesize under uniform light conditions in an atmosphere containing carbon dioxide which was tagged with the radioisotope of carbon, carbon-14 (^{14}C). Subsequent measurement of radioactivity incorporated into the leaves served as a measure of photosynthetic activity. The fate of the assimilated carbon as a function of leaf age was determined by measuring the amount of radioactivity present in the different categories of compounds present in the leaves. Detached leaves were used in this investigation in order to prevent redistribution of assimilated carbon through translocation from the site of assimilation to other locations in the tea bush. In this way a measure of the photosynthetic capabilities of the individual leaves was obtained. The results showed that, when illuminated with the same amount of light, the photosynthetic efficiency (that is the amount of photosynthetic activity per unit weight of leaf tissue) increases in leaves until they are about one half of their mature size. However, the photosynthetic capacity (that is the total amount of photosynthetic activity per leaf) increases as the size of the leaves increase. These results suggest that the mature leaves (maintenance foliage) on tea bushes are important sources of carbon compounds for these plants.

It was found that the leaf age had a marked effect on the type of compounds elaborated from photosynthetically assimilated carbon. In immature leaves a relatively large proportion of the assimilated carbon was incorporated into flavanols (catechins) and compounds which would be utilized *in situ* (such as amino acids, organic acids, *etc*). On the other hand, in mature leaves the majority of the assimilated carbon was found to be incorporated into easily translocatable substances such as sugars. These results also suggest that the mature foliage on tea bushes nurture the growing (immature) parts of the plant by supplying them with carbon compounds.

Lines of research which should help to verify the indications obtained in this investigation are pointed out.

Introduction

One important objective of a tea grower is to promote a continuous production of new flush[1] (young shoot tips), and to do this in such a way as to maximize the potential of this flush to make quality tea. A requisite for sustained growth by tea plants, or any living organism, is a continuous supply of food materials such as carbon compounds (*eg* carbohydrates), inorganic salts and water. Successful cultivation of any crop is, therefore, dependent on ensuring an adequate supply of these materials to the regions of the plant producing the harvestable tissues.

It is well known that the carbon compounds in green plants originate almost entirely through assimilation of atmospheric carbon dioxide by the process called photosynthesis and that this process takes place only in the green parts of the plant in the presence of light. In spite of the recognized importance of the problem, very little is known about the relative importance of the various green parts (leaves) of the tea plant in the photosynthetic assimilation process.

The investigation described in this paper has been undertaken for the purpose of determining the potential of various green parts of the tea plant to carry out photosynthesis. Radioactive carbon (^{14}C) has been used in these investigations and this has permitted us to determine the type of compounds formed from the photosynthetically assimilated carbon dioxide as a function of leaf age. Detached leaves have been used in this study intentionally so that translocation effects would be eliminated. In other words, the purpose has been to determine the capabilities of the leaves themselves to assimilate carbon dioxide photosynthetically. Certain tentative conclusions as to the relative role of the various leaves in the nutrition of the developing flush made on the basis of the experimental results are discussed.

It is to be hoped that the results of this and similar future investigations will be useful in helping to formulate a scientific basis for such cultural practices as pruning and plucking. Further, it is hoped that they will assist in our efforts to define the potential of flush to make quality teas in chemical terms.

Definitions

The following definitions will be used throughout this paper: *Photosynthetic capacity*—The overall ability of a plant part to assimilate carbon dioxide photosynthetically under specified conditions. Photosynthetic capacity was measured as the counts/100 seconds assimilated per plant part when allowed to photosynthesize in an atmosphere containing $^{14}CO_2$ for 1 hour (illumination at 9 klux). *Photosynthetic efficiency*—The ability of a plant part to photosynthetically assimilate carbon dioxide per unit weight of plant tissue. Again, photosynthetic efficiency was determined under the set of conditions described above.

Materials and methods

Source of plant material

All plant material came from the clone TRI 2024 growing in the field (1500 m elevation). $^{14}CO_2$ treatments commenced within $1\frac{1}{2}$ hours of removal of the plant material from the field.

$^{14}CO_2$ treatment

The plant material was separated into the parts to be treated with $^{14}CO_2$ in the laboratory. The parts were then suspended in the $^{14}CO_2$ treatment chamber (Figure 1) by hanging them on a wire frame inside the chamber. A thin layer of water on the bottom of the chamber humidified the atmosphere within the chamber and prevented wilting. Leaves could be kept inside this chamber for at least 72 hours without showing visible signs of deterioration.

The $Ba^{14}CO_3$ (about 160 μc of ^{14}C) was placed in a shallow beaker just beneath the tip of the acid delivery tube (B in Figure 1). The chamber was sealed and it was placed in a light chamber (9 klux) at 30°C. $^{14}CO_2$ was generated by delivering 3 ml of 1 N lactic acid onto the $Ba^{14}CO_3$. The atomsphere within the treatment chamber was mixed thoroughly by operating the aspirator (C in Figure 1) for 5 min initially and for 1 min every 5 min thereafter. $^{14}CO_2$ treatment was terminated after 1 hour by delivering 5 ml of 2N NaOH into the chamber through the acid delivery tube. The system was aspirated for an additional 5 min before the chamber was opened.

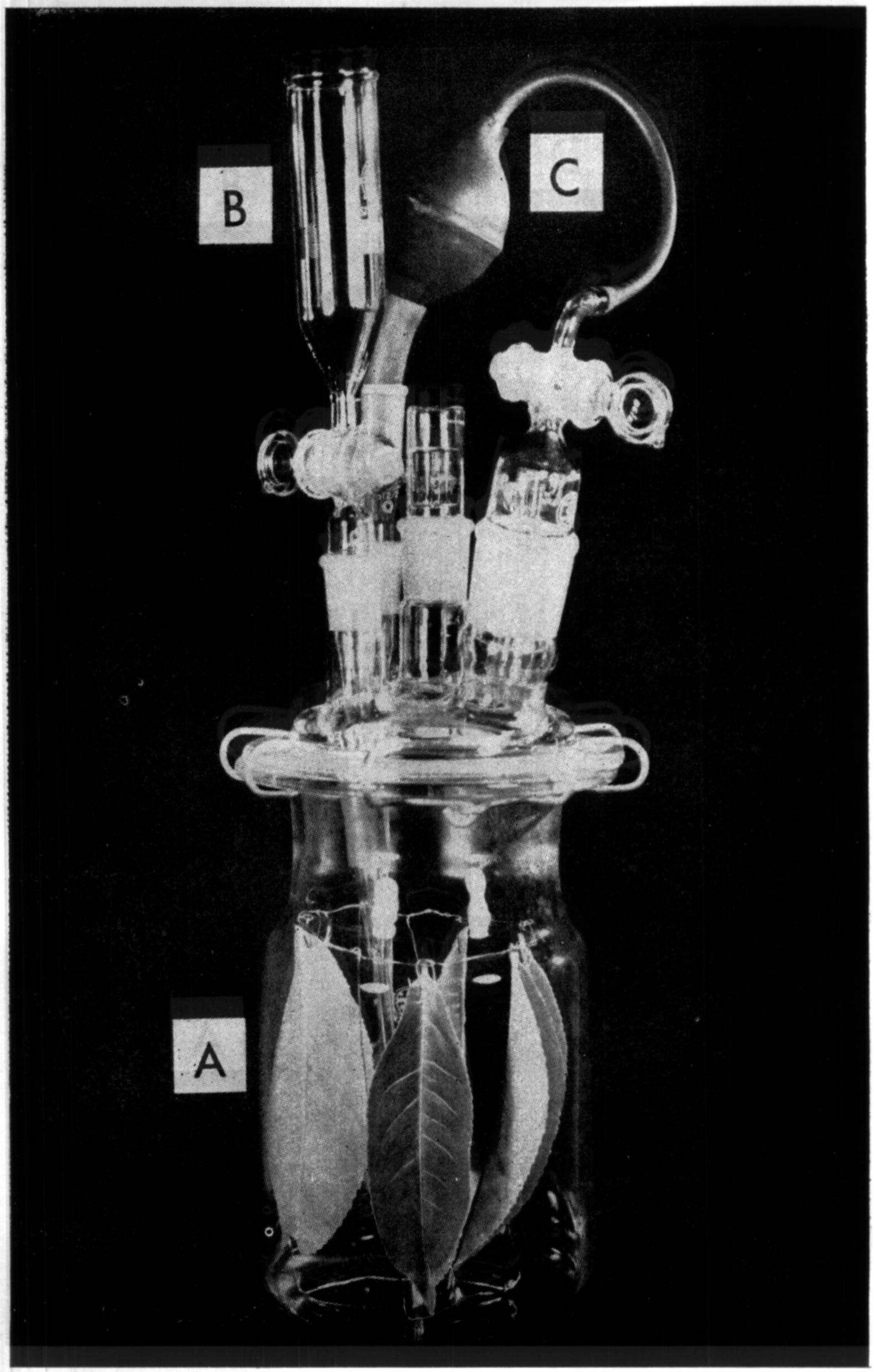

FIGURE 1—$^{14}CO_2$ *treatment chamber—A—Chamber containing detached leaves suspended on a wire frame—B—Acid delivery tube—C—Aspirator system used to circulate air within the treatment chamber.*

Extraction of $^{14}CO_2$ *treated plant material and fractionation of extracts*

$^{14}CO_2$ treated plant material was killed within 10 min of terminating the treatment by placing it in boiling distilled water for 3 min. The tissues were then blended for 10 min in about 10 volumes of distilled water. The macerates were centrifuged and the residues were re-extracted 3 times with about 5 volumes of distilled water. The washed residues were dried for determination of ^{14}C content after wet ashing with the Van Slyke-Folch reagent (Van Slyke *et al* 1951) and conversion to $Ba^{14}CO_3$ (Baker *et al* 1954).

The combined aqueous extracts (the crude soluble fractions) were fractionated as follows:

1 – Extracts were treated successively with 0.5 and 0.1 g of Polyclar AT (an insoluble cross-linked polyvinylpyrrolidone manufactured by Antara Chemicals Division of General Aniline & Film Corp, New York, USA). This treatment removes phenolic compounds, and other unidentified compounds. This fraction was called the '*other*' fraction.

2 – The aqueous extract was then extracted with chloroform (5 times with 1/5th volume) to remove caffeine and closely related compounds.

3 – The aqueous extract was next passed through a cation exchange column (Amberlite CG 120, H^+ form) followed by an anion exchange column (Amberlite CG 4B, OH^- form). The effluent from these columns was called the *neutral* fraction and it contained the carbohydrates and related compounds. The cation exchange column was eluted with 10% HCl and the eluate which contained the amino acids and other basic compounds was called the *cationic* fraction. The anion exchange column was eluted with 2N NH_4OH and the eluate which contained the organic acids and related compounds was called the *anionic* fraction. This procedure is summarized below.

OUTLINE OF FRACTIONATION PROCEDURE

$^{14}CO_2$ TREATED LEAF

(Kill tissue, extract with water)

——> INSOLUBLE RESIDUE

V

CRUDE SOLUBLE FRACTION

(Treat with Polyclar AT)

——> Polyclar AT with absorbed *OTHER* substances (Phenolics, *etc*)

(Extract with chloroform)

——> Chloroform extract (*CAFFEINE, etc*)

(Pass through cation exchange column)

——> Cation exchange column eluate: *CATIONIC FRACTION* (amino acids, *etc*)

(Pass through anion exchange column)

——> Anion exchange column eluate: *ANIONIC FRACTION* (organic acids, *etc*)

V

Effluent from ion exchange columns: *NEUTRAL FRACTION* (Carbohydrates, *etc*)

TABLE 1—*Description of material treated with* $^{14}CO_2$

A—Parts of long tea shoots (Figure 2)				B—Parts of shoots from tea bushes in plucking (Figure 3)			
Plant part	Approximate age in days from unfolding	Fresh weight/ part (g)	% dry matter	Plant part	Approximate age in days from unfolding	Fresh weight/ part (g)	% dry matter
Bud	—	0.076	25.9	Bud	—	0.064	23.2
1st leaf	0—3	0.193	25.4	1st leaf	0—3	0.111	25.4
2nd leaf	9—12	0.409	24.8	2nd leaf	9—12	0.202	24.7
3rd leaf	18—21	0.801	28.4	3rd leaf	18—21	0.240	24.8
4th leaf	27—30	1.05	33.7	Unlignified stem	—	0.292	14.7
5th leaf	36—39	1.19	35.2	Maintenance leaf	100—120	0.656	27.4
7th leaf	54—60	1.33	35.6	'Old leaf'	200—400	0.659	36.1
Unlignified stem	—	0.38	16.4				

Flavanols were separated from other compounds in the crude soluble fraction by paper chromatography (Roberts 1962) and they were collected by eluting them from the chromatograms with water.

Determination of radioactivity

All radioactivity was determined using a thin end window Geiger-Muller tube. Samples of $Ba^{14}CO_3$ obtained from wet ashing of insoluble residues were plated onto filter paper which was placed on a planchet and counted as layers of finite thickness. These counts were corrected to infinite thinness using a published correction table (Calvin *et al* 1949). Aliquots of samples in solution were plated directly onto planchets and counted as infinitely thin layers.

Results

Pattern of $^{14}CO_2$ assimilation by various parts of 'long' tea shoots

This type of shoot is found on tea bushes which are in an active state of free growth as in the case of bushes which are allowed to grow out for the production of material for leaf cuttings used for vegetative propagation (Visser & Kehl 1958). A shoot typical of those used in this experiment is shown in Figure 2. The material is further described in Table 1. The age of the leaves was estimated from data given by Pethiyagoda (1964).

It is important to point out at this stage that the high level of incorporation of photosynthetically assimilated carbon into the insoluble residue after short periods of exposure to $^{14}CO_2$ (70 min) is due to an artifact. This artifact is brought about by the polyphenolic compounds which are present at high levels in these tissues. They constitute from 20 to 35% of their dry weight (*cf* Stahl 1962) and they cause a considerable amount of the soluble substances present in tea leaves to precipitate during extraction when usual techniques are used (see Sanderson 1965a). Because of this artifact, the total amounts of radioactivity assimilated (Tables 2 & 5) are considered to be more accurate indicators of photosynthetic activity than either of their components, namely the soluble fractions and the insoluble residues.

TABLE 2—*Photosynthetic capacity and photosynthetic efficiency of various parts of long shoots (Figure 2)*

Description of sample	Photosynthetic capacity (10^{-3} counts/100sec/part)			Photosynthetic efficiency (10^{-3} counts/100 sec/g dry wt)		
	Soluble fraction	Insoluble residue	Total	Soluble fraction	Insoluble residue	Total
Bud	3.0	4.1	7.1	151	203	362
1st leaf	45	35	80	914	720	1634
2nd leaf	377	154	531	4150	1700	5851
3rd leaf	1080	845	1925	5400	4200	9600
4th leaf	1705	1070	2775	5440	3410	8850
5th leaf	2190	2265	4455	4850	5030	9880
7th leaf	2930	2690	5620	5590	5120	10710
Unlignified stem	15	12	27	238	189	427

Table 3—*Distribution of assimilated carbon in the soluble fractions of parts of long shoots (Figure 2)*

Description of sample	Neutral fraction		Cationic fraction		Anionic fraction		Caffeine	Others		Total
	(10^{-3} counts/100 sec/plant part)									
Bud	0.7	(24)*	0.9	(31)	0.9	(31)	0.005	0.5	(14)	3.0
1st leaf	9.9	(22)	8.6	(19)	10	(22)	0.084	16	(27)	45
2nd leaf	131	(35)	42	(11)	74	(20)	0.258	130	(34)	377
3rd leaf	386	(36)	90	(8)	235	(22)	0.440	365	(34)	1076
4th leaf	707	(41)	107	(6)	252	(15)	0.467	639	(38)	1705
5th leaf	745	(34)	105	(5)	230	(11)	0.365	1106	(50)	2186
7th leaf	1106	(38)	89	(3)	175	(6)	0.352	1563	(53)	2933
Unlignified stem	3.2	(21)	3.3	(22)	4.1	(27)	0.038	4.4	(30)	15

*Numbers in parenthesis show counts assimilated as % of the total soluble counts assimilated

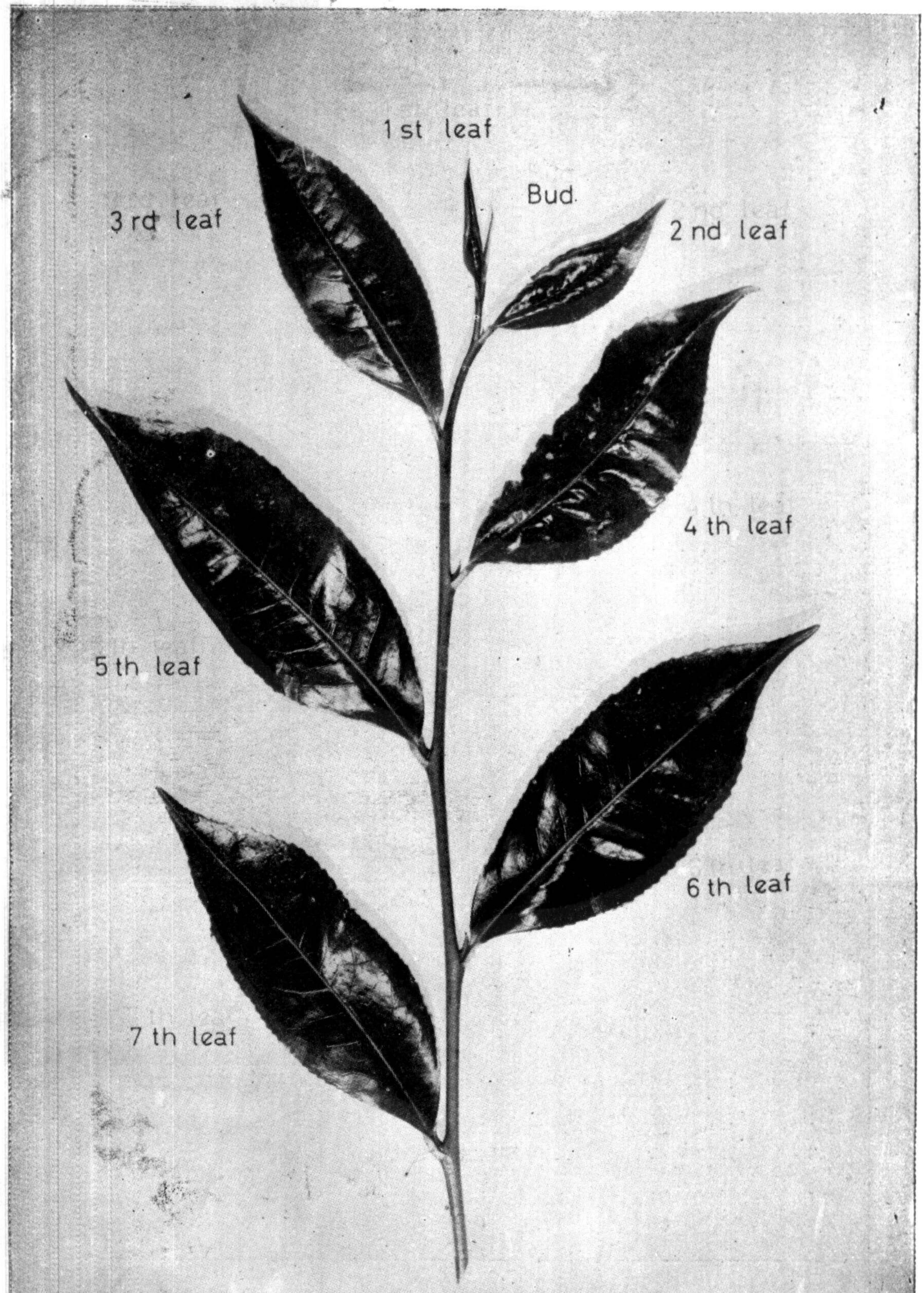

FIGURE 2—*Long tea shoot—This shoot is typical of shoots from tea plants (especially clone TRI 2024) allowed to grow out freely for the production of cuttings for use in vegetative propagation*

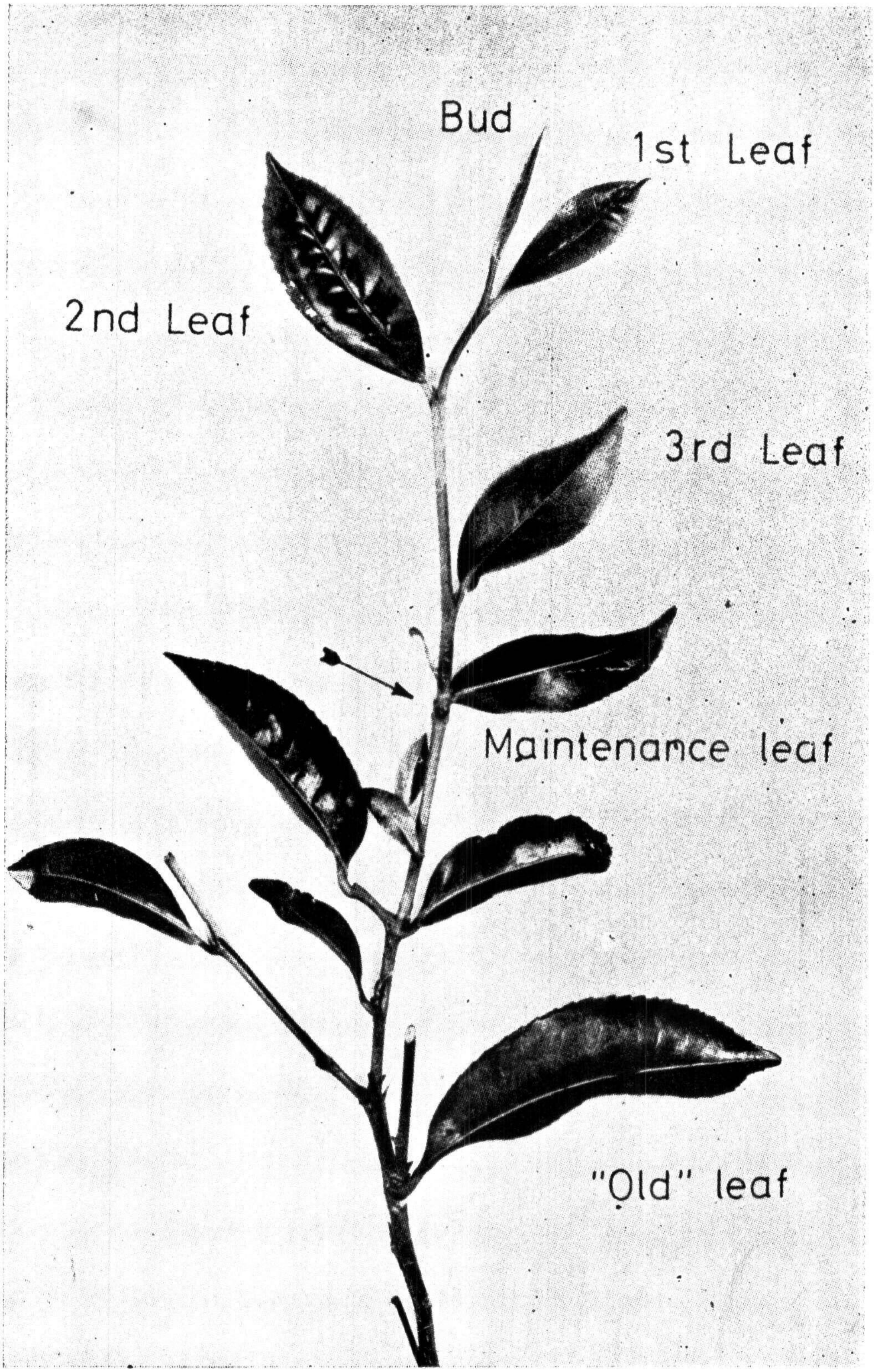

FIGURE 3—*Shoot from tea bush in plucking—This shoot is typical of shoots on tea bushes (especially clone TRI* 2024) *which are being cropped (plucked)—The arrow indicates the leaf axil in which a new shoot tip has arisen—These new shoot tips (called flush) are the part of the tea plant which is harvested—The older maintenance leaf is not useable in orthodox tea manufacture and it is left on the bush to support new growth—The so called 'old' leaves are former maintenance leaves which have become buried within the bush and which are normally heavily shaded*

The results of this experiment (Table 2) show that the photosynthetic capacity of leaves increases with leaf age at least up to the age of the 7th leaf which would be about 63 days old. However, the photosynthetic efficiency increases from the bud only up to about the 4th leaf which would be about 36 days old. The unlignified stem which extends from the base of the bud to the 3rd node makes only a small contribution to the plant's photosynthetic activity.

The distribution of photosynthetically assimilated carbon after a 1 hour period of photosynthesis was investigated. For this purpose the crude soluble fraction was fractionated into the 5 categories described above and the radioactivity in each fraction was determined (Table 3). The following differences in the fate of this carbon in leaves of different ages are noteworthy: (*a*) The *neutral* fraction (carbohydrates *etc*) tends to contain an increasing proportion of the total soluble counts as the age of the leaves increase. (*b*) The proportion of the assimilated carbon in the *cationic* fraction (amino acids *etc*) and the *anionic* fraction (organic acids *etc*) decreases as the age of the plant part increases. (*c*) The amount of caffeine synthesized from assimilated carbon increases to the 3rd or the 4th leaf after which it tends to decrease. (*d*) The proportion of the soluble assimilated carbon in the '*other*' fraction (phenolics *etc*) increases as the age of the plant part increases. The probable significance of this will be discussed below.

TABLE 4—*Synthesis of flavanols from photosynthetically assimilated carbon in long tea shoots (Figure 2)*

Description of sample	AMOUNT OF CARBON ASSIMILATED INTO FLAVANOLS counts/part	counts/g dry wt tissue	% of 'other' fraction in Table 3	% of total soluble fraction in Table 3
Bud	300	15,400	61	10
1st leaf	3,300	67,000	14	7.3
2nd leaf	33,500	367,000	26	8.9
3rd leaf	66,700	335,000	18	6.2
4th leaf	59,200	189,000	9.3	3.5
5th leaf	14,100	31,200	1.2	0.6
7th leaf	6,700	12,600	0.4	0.2
Unlignified stem	450	6,700	10	2.9

The synthesis of flavanols (catechins) increases very markedly from the bud to the 3rd or 4th leaf after which it decreases very rapidly (Table 4). The percentage of the assimilated carbon found in the flavanols after 70 min of photosynthesis, however, decreases somewhat regularly with increasing leaf age. The rather low percentage of the assimilated carbon in the 'other' fraction accounted for by flavanols was surprising. The compounds in this fraction which do contain the assimilated carbon are largely unknown at the present time; additional research on this point is needed.

Pattern of $^{14}CO_2$ assimilation by various parts of shoots from tea bushes in plucking

This type of shoot is found on tea bushes which are being continuously cropped or plucked. A shoot typical of those used in this experiment is shown in Figure 3. The samples are further described in Table 1. The results of this experiment (Tables 5, 6 & 7) are, in general, similar to those for parts of long tea shoots described above. The following additional points, however, are noteworthy: (*a*) The photosynthetic efficiency of the mature leaves, namely the maintenance leaves and the 'old' leaves, is nearly as great as the most efficient of the immature leaves, namely the 3rd leaves, and their capacity for photosynthesis is considerably greater than any of the immature leaves because of their greater size (Table 5). (*b*) The increase in the amount of carbon assimilated into the *neutral* and the *other* fractions in mature leaves is especially marked in these shoots (Table 6). The probable importance of this in relation to the nutrition of the flush is discussed below.

TABLE 5—*Photosynthetic capacity and photosynthetic efficiency of various parts of shoots from bushes in plucking (Figure 3)*

Description of sample	Photosynthetic capacity (10^{-3} counts/100 sec/part)			Photosynthetic efficiency (10^{-3} counts/100 sec/g dry wt)		
	Soluble fraction	Insoluble residue	Total	Soluble fraction	Insoluble residue	Total
Bud	4.5	2.6	7.1	303	175	478
1st leaf	21	8.3	29	745	294	1039
2nd leaf	41	14	55	823	281	1104
3rd leaf	77	22	99	1290	370	1660
Unlignified stem	18	7.5	25	420	175	595
Maintenance leaf	200	40	240	1110	222	1332
'Old' leaf	258	21	279	1090	88	1178

Discussion

Perhaps the most important finding in this investigation is the high photosynthetic efficiency of mature tea leaves (Tables 2 & 5). This, coupled with their relatively large size gives them a considerably greater photosynthetic capacity than the immature leaves. Barua (1960) measured rates of photosynthetic assimilation by detached tea leaves by measuring the depletion of atmospheric CO_2 in closed jars and he also found that tea leaves do not reach their maximal photosynthetic efficiency until they are about one half of their mature size. These findings would appear to give a sound basis for the contention that the maintenance foliage (mature leaves) is important in supporting the growth of the flush (Barua 1961). On the other hand, the results of recent experiments in Ceylon (Pethiyagoda 1965) have indicated that, at least under the conditions of the experiment, removal of all mature leaves below

TABLE 6—*Distribution of assimilated carbon in the soluble fractions of parts of shoots from bush in plucking* (*Figure 3*)

Description of sample	Neutral fraction		Cationic fraction		Anionic fraction		Caffeine (10^{-3} counts/100 sec/plant part)	Others		Total
Bud	0.7	(15)*	1.5	(33)	1.2	(27)	0.008	1.1	(25)	4.5
1st leaf	5.6	(26)	4.6	(22)	4.7	(22)	0.037	7.1	(30)	21
2nd leaf	13	(32)	7.6	(18)	8.6	(21)	0.071	12	(29)	41
3rd leaf	31	(40)	9.2	(12)	16	(21)	0.108	21	(27)	77
Unlignified stem	8.2	(44)	2.2	(12)	3.4	(19)	0.012	4	(25)	18
Maintenance leaf	82	(41)	9.2	(5)	9.1	(5)	0.009	100	(49)	200
'Old' leaf	145	(56)	8.5	(3)	5.5	(2)	0.017	99	(39)	258

*Numbers in parenthesis show counts assimilated as % of the total soluble counts assimilated.

TABLE 7—*Synthesis of flavanols from photosynthetically assimilated carbon in shoots from tea bushes in plucking* (*Figure 3*)

Description of sample	counts/part	counts/g dry wt tissue	% of 'other' fraction in Table 6	% of total soluble fraction in Table 6
	AMOUNT OF CARBON ASSIMILATED INTO FLAVANOLS			
Bud	250	16,800	22	5.4
1st leaf	1,150	40,800	16	5.5
2nd leaf	1,850	37,200	15	4.6
3rd leaf	4,350	73,000	21	5.6
Unlignified stem	400	9,300	10	2.2
Maintenance leaf	1,480	6,700	1.7	0.6
'Old' leaf	1,450	6,950	1.7	0.6

the plucking table has only a slight depressing effect on yield. Reconciliation of the results of these two markedly different types of experiments may be tied in with the amount of light which actually gets to the mature leaves. The results of the experiments reported in this paper clearly show that, given equal light treatment, mature tea leaves are capable of more photosynthetic activity than immature leaves. Hadfield (1963; 1964) has, however, reported data showing that only about 14% of the incident light penetrates to a depth of 10 cm below the plucking table level in typical fields of tea in Assam. Additional evidence for the importance of incident light intensity is given by Barua (1964) who studied the effect of light intensity on assimilation rates in detached tea leaves. He found that the rate of photosynthesis increased as the light intensity increases (at least up to 32 klux). He also found that there were marked differences between clones of tea and their response to increasing light intensity which might be of importance in comparing the results of different experiments. Clearly, additional research is required for a full understanding of the problem.

The distribution of photosynthetically assimilated carbon between the several categories of soluble compounds in leaves of different ages (Tables 3 & 6) is also suggestive of their respective roles in the overall nutrition of the tea plant. That is, in immature leaves the major part of the assimilated carbon is found in the *cationic* and the *anionic* fractions which contain the compounds most likely to be used *in situ* in growing tissues (*ie* organic acids and amino acids to provide respiratory energy and structural materials for synthesis of cellular ground substances), whereas in mature leaves only a small proportion of the assimilated carbon is found in these fractions. On the other hand, the *neutral* and the '*other*' fractions, which contain the most readily translocated compounds (*ie* carbohydrates), contain an increasing proportion of the assimilated carbon as the age of the leaf increases. It should be possible to verify the validity of these suppositions by investigation of the translocation patterns in mature tea bushes in plucking using radiochemical techinques.

The increasing proportion of assimilated carbon which is found in the '*other*' fraction (Tables 3 & 6) as the leaves age was surprising. The Polyclar AT treatment has been shown to be very effective in removing polyphenolic compounds from plant extracts (Sanderson 1965a) and there are indications that this treatment does not remove carbohydrates and that it removes only negligible amounts of amino acids believed to be phenolic amino acids. If it is true that the Polyclar AT treatment removes polyphenolic compounds and only negligible amounts of other compounds, then it must be concluded that the mature leaves are important sites of synthesis of phenolic compounds. However, it was found that the flavanols themselves (Tables 4 & 7), which are the major phenolic compounds present in the flush and which are so important in tea manufacture (Roberts 1962; Sanderson 1965b), contain a rapidly diminishing proportion of the assimilated carbon as the leaves age (Table 4 & 7) in agreement with the results of experiments by Zaprometov (1963). It may be that the phenolic compounds other than flavanols are the main ones synthesized by mature leaves in contrast to the situation in immature leaves. In any case, the results of the present investigation are supported by recent findings which suggest that the enzymes involved in the biosynthesis of phenolic compounds are present in mature tea leaves at levels which surpass those in immature leaves (Sanderson 1966). Again, additional research is required to determine more exactly the composition of the 'other' soluble fraction as defined in this investigation before the significance of these results can be accurately assessed.

Research is now in progress which is designed to answer the following questions:

1 – Is the carbon dioxide which is photosynthetically assimilated by the maintenance foliage actually translocated into the developing flush and, if it is, is it actually used to nurture these tissues?

2 – What is the relative contribution of the maintenance foliage and the flush itself to the elaboration of the chemical constituents of the flush which are known to be important in tea manufacture; especially the flavanols.

Summary and conclusions

The results of this investigation show clearly that mature tea leaves are as efficient in photosynthetically assimilating carbon dioxide as the most efficient immature leaves, and, because of their relatively large size, they have a considerably greater capacity than the immature leaves. The results further suggest that the mature leaves are important in nurturing the development of new shoots; the harvestable material. Suggestions are made for future research which should help to corroborate and amplify the results of this investigation.

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